

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | |
|---|-----------|---|
| (51) International Patent Classification ⁵: G01N 33/543, C12Q 1/00 | A1 | (11) International Publication Number: WO 94/24561 (43) International Publication Date: 27 October 1994 (27.10.94) |
| (21) International Application Number: PCT/SE94/00343 (22) International Filing Date: 18 April 1994 (18.04.94) (30) Priority Data: 9301270-6 19 April 1993 (19.04.93) SE (71)(72) Applicants and Inventors: NILSSON, Kurt [SE/SE]; Andjaktst. 6, S-226 53 Lund (SE). MANDENIUS, Carl-Fredrik [SE/SE]; Strömkarlsv. 36, S-141 42 Huddinge (SE). | | (81) Designated States: CA, CZ, JP, RU, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> <i>In English translation (filed in Swedish).</i> |
| (54) Title: IMMOBILIZED CARBOHYDRATE BIOSENSOR (57) Abstract The present application refers to a biosensor in which an immobilized carbohydrate or a derivative thereof is used to generate a detectable signal when a protein, a virus or a cell is bound to the carbohydrate surface. The sensor is an optical sensor, a piezoelectric sensor, an electrochemical electrode or a thermistor. A method of binding carbohydrates to a gold surface is also described. | | |

BEST AVAILABLE COPY

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | |
|----|--------------------------|----|---------------------------------------|----|--------------------------|
| AT | Austria | GB | United Kingdom | MR | Mauritania |
| AU | Australia | GE | Georgia | MW | Malawi |
| BB | Barbados | GN | Guinea | NE | Niger |
| BE | Belgium | GR | Greece | NL | Netherlands |
| BF | Burkina Faso | HU | Hungary | NO | Norway |
| BG | Bulgaria | IE | Ireland | NZ | New Zealand |
| BJ | Benin | IT | Italy | PL | Poland |
| BR | Brazil | JP | Japan | PT | Portugal |
| BY | Belarus | KE | Kenya | RO | Romania |
| CA | Canada | KG | Kyrgyzstan | RU | Russian Federation |
| CF | Central African Republic | KP | Democratic People's Republic of Korea | SD | Sudan |
| CG | Congo | KR | Republic of Korea | SE | Sweden |
| CH | Switzerland | KZ | Kazakhstan | SI | Slovenia |
| CI | Côte d'Ivoire | LI | Liechtenstein | SK | Slovakia |
| CM | Cameroon | LK | Sri Lanka | SN | Senegal |
| CN | China | LU | Luxembourg | TD | Chad |
| CS | Czechoslovakia | LV | Latvia | TG | Togo |
| CZ | Czech Republic | MC | Monaco | TJ | Tajikistan |
| DE | Germany | MD | Republic of Moldova | TT | Trinidad and Tobago |
| DK | Denmark | MG | Madagascar | UA | Ukraine |
| ES | Spain | ML | Mali | US | United States of America |
| FI | Finland | MN | Mongolia | UZ | Uzbekistan |
| FR | France | | | VN | Viet Nam |
| GA | Gabon | | | | |

Immobilized carbohydrate biosensor

The present invention relates to a biosensor in which a carbohydrate or a derivative thereof is used to generate a detectable signal via the specific binding of a protein, a virus or a cell.

Background.

Biosensors are characterised by a physical or chemical signal transducer, which response is activated by a specific interaction between a biochemical structure (which directly or indirectly has been bound to the transducer) and one or several analytes.

Biosensors are used to detect the analyte/analytes and in certain cases also for quantification of the analyte/analytes.

The advantages of the biosensor are that a physical or chemical transducer has been made specific so that a general physical or chemical parameter (e.g. temperature, pH, optical density) can be used for the detection of one specific substance in a complex mixture of non-specific substances.

The limitations of the biosensor are the specificity of the biochemical structure bound to the transducer, the range of specificity and stability, and, that the transducer signal has to be made independent of the background changes in the parameter that the transducer is measuring. In Methods of Enzymology, volume 137, several articles are describing different aspects of biosensors.

Definitions.

Biosensor - physical or chemical signal transducer, e.g. photometer, chemical electrode, temperature or pressure signal transducer, which directly or indirectly has been connected with a biochemical structure. In previous biosensors one has preferentially used an enzyme, a specific protein or antibody as the biochemical structure and in this way the biosensors have been given the property of being able to detect substances which specifically bind to the biochemical structure in a qualitative or quantitative way.

2.

Reflection measurement - measurement of the intensity of light reflected from a surface where the properties of the surface influences the reflection, e.g. biomolecules which change the refraction index of the surface.

Polarisation measurement - measurement of the polarisation of polarised light, usually as the angle of polarisation, which is depending on the binding of biomolecules, virus or cells.

Surface plasmon spectroscopy - optical physical measurement technique which utilise the surface plasmon condition of thin metal surfaces, which can be used to measure small changes of refraction index with high sensitivity, e.g. as caused by the presence of biomolecules on the surface.

Ellipsometry - optical physical measurement technique which can be used to measure small changes of refraction index at surfaces with high sensitivity, by measuring changes in ellipticity of polarised light, e.g. as caused by the presence of biomolecules on the surface.

Piezoelectric crystal - crystal which frequency can be influenced by changes of mass or pressure which can be measured electrically, for example the change of mass caused by the presence of biomolecule(s), virus or cell(s) bound to the crystal surface.

Electrochemical electrode - measuring device which generates an electrical signal caused by an electrochemical reaction at the electrode which is related to a chemical parameter, e.g. pH, pO_2 , pCO_2 , the values of which can vary because of the presence of analyte(s) in a sample specific for a compound bound to the measuring device.

Thermistor - electrical resistance device which changes resistance with the temperature; biochemical reactions are characterised by e.g. specific values of heat consumption/formation, which can be registered via the thermistor.

A large amount of the carbohydrate sequences present in glycoproteins or in glycolipids, and usually also smaller fragments of these sequences, have shown biospecific binding to proteins, virus or cells.

3.

The present invention describes a biosensor where this specificity is used for determination of such a component in a sample. The invention is characterised by that the carbohydrate or a derivative thereof is bound to a surface in the biosensor.

As carbohydrate, one can use fragments (oligosaccharides) of the carbohydrate sequences found in glycoproteins or in glycolipids and one can also use smaller fragments of these sequences, i.e. disaccharide, trisaccharide, tetrasaccharide or a pentasaccharide, because this size usually is sufficient for the oligosaccharide to bind a protein, virus or a cell in a biospecific manner. A review of such carbohydrate sequences can be found in e.g. Chemistry and Physics of Lipids, vol. 42, p. 153-172, 1986, and in Ann. Rev. Biochem., vol. 58, p. 309-350.

The oligosaccharide is usually modified in the reducing end with an aglycon, which is composed of a glycosidically bound organic group which is suitable for binding to the surface in the biosensor. Examples of aglycons are OEtSEtCONHNH_2 , -OEtSPhNH_2 , etc. The binding to the surface in the biosensor can be done directly or via a proteins, e.g. bovine serum albumine or via a chemical structure which has been adsorbed or which has been covalently bound to the surface. Such a chemical structure can contain reactive organic groups such as carboxyl-, sulfonate, cyanate, epoxy-, aldehyde groups or other groups suitable for chemical conjugation with for example an amine or thiol group in the aglycon.

More specific examples of analytes which can be analysed with biosensor according to the present invention are lectins, antibodies against carbohydrates, pathogenic virus or bacteria, such as urinary tract bacteria (e.g. P-fimbriated *E. coli*) or pathogens of the respiratory tract, and bacteria which cause infections/diarrhea in in gastrointestinal tract.

Non-limiting examples of carbohydrate structures of interest and which can be used in the form of a carbohydrate derivative in a biosensor according to the invention, are monosaccharides, disaccharides, trisaccharides and higher oligosaccharides which show biological activity or which has the ability to specifically bind one or more biomolecules or a group of biomolecules. Examples of biomolecules are other saccharides, peptides and proteins. Examples of such carbohydrate sequences are the blood group determinants (for example A, B, H, Lewis-a, Lewis-b, Lewis-x, Lewis-y), cancer-associated carbohydrate sequences,

4.

carbohydrate sequences (often di-, tri- or tetrasaccharides) which bind to pathogenic bacteria/toxins or virus of for example the respiratory, the gastro-intestinal or the urinary tract, carbohydrate sequences which bind to proteins/cells/white blood cells associated with inflammatory reactions (for example selectin-carbohydrate reactions).

These and other carbohydrate structures which can be used in a biosensor according to the present invention often contain one or more of the following monosaccharides (or a derivative or an analog of any of these) which are α - or β -glycosidically bound: hexosamine, fucose, mannose, glucose, N-acetyl-glucosamine, N-acetyl-galactosamine, xylose, galactose, or another monosaccharide. These components are usually present in for example pyranose or furanose form.

Examples of carbohydrate derivatives are derivatives where the carbohydrate or a derivative or an analog, are modified in the reducing end with an O-, N-, C- or S-glycosidically bound aglycon which can be an aliphatic or an aromatic compound, an amino acid-, peptide- or protein molecule or a derivative thereof. The aglycon part can thus be composed of for example an O-, N-, C- or S-glycosidically bound aliphatic or aromatic compound which is bound to an amino acid-, peptide- or protein molecule or a derivative thereof. Examples of carbohydrate derivatives which can be used according to the invention are structures in which one or more of the hydroxyl groups in the carbohydrate, in addition to or instead of the hydroxyl group in the reducing end of the carbohydrate part, have been modified with an organic or inorganic group. This can be of interest, for example to increase/modify the biological activity or to facilitate the binding to the biosensor surface according to the invention.

The aglycon part or another group can be used for adsorption or covalent binding of the carbohydrate derivative to the surface of the biosensor and can be used in the invention as a spacer molecule between the biosensor surface and the carbohydrate part to minimise sterical hindrance in the binding of the analyte to the carbohydrate part in the biosensor according to the invention.

The aliphatic or aromatic compound in the aglycon can for example consist of structures of the type -R-X, where R- consists of an organic compound, for

5.

example an alkyl chain of the type $(-CH_2)_n-$, in which n is an integer, e.g. in the interval 2 to 8, or is composed of an aromatic group-containing structure, and where $-X$ is for example a structure of the type $-S-$, amide $(-NH-CO-$ or $CO-NH-)$, amine $(-NH-)$, a $-N=N-$ group or another group suitable for binding to the surface in the biosensor or to a protein (i.e. in the latter case the carbohydrate derivative is a neoglycoprotein). When the carbohydrate derivative is a neoglycoprotein R can be used as a spacer between the protein part and the carbohydrate part. The spacer often has a functional part $(-X-$ above) which has been used in the binding to the protein.

Suitable spacer and functional group is chosen by the person skilled in the art and does not limit the scope of the invention.

The carbohydrate derivative can also, according to the invention, be composed of a natural, *in vitro* isolated glycoprotein or a recombinant glycoprotein or a glycopeptide. This type of derivative can be adsorbed to the surface in the biosensor, for example a gold- or silica surface or another surface which adsorbs proteins, lipids or peptides.

In the case a covalent binding is desired one can, as in the case when the carbohydrate derivative is a neoglycoprotein, use for example the protein part's amino-, carboxyl-, or thiol groups for binding to the surface in the biosensor. This (as for the synthesis of the neoglycoprotein from carbohydrate spacer and protein) can be done with the standard techniques which normally are used for modification of proteins and for immobilisation of proteins to solid supports (see for example methods mentioned in Methods of Enzymology, volumes 44, 102, and 135), and the choice of suitable technology is made by the person skilled in the art in every specific case. Examples of methods are coupling or activation of carboxyl groups with carbodiimide reagents, N-hydroxysuccinimide reagents, of hydroxyl groups with CNBr, sulphonyl chloride (tresyl chloride, tosyl chloride), divinyl sulphone, periodate (gives aldehyde groups), thiol groups are activated with thiol reagents of the type SPDP, etc.

As examples of surfaces according to the invention may be mentioned:

Carbohydrate-R-X-Biosensor surface or

6.

Carbohydrate-R-X-Protein-Biosensor surface

where Carbohydrate, R and X have been exemplified above. X and Protein can be directly adsorbed on the Biosensor surface above, but between X and Biosensor surface above and between Protein and Biosensor surface above can also a chemical group be present, for example a $-\text{CO}-\text{CH}_2\text{CH}_2-\text{S}-$ group, i.e. for example:

**Carbohydrate-R-NH-CO-CH₂-CH₂-S-Biosensor surface or
Carbohydrate-R-X-Protein-NH-CO-CH₂-CH₂-S-Biosensor surface.**

As protein one can use for example bovine serum albumin, but all for the application suitable types of proteins can be used in the carbohydrate derivative-based biosensor according to the invention.

The biosensor according to the invention can be designed in a variety of configurations. Examples are:

- a) planar carbohydrate surface which easily can be contacted with the sample, for example a surface designed as a dipstick, this surface can be placed in a measuring device for optical reflectance measurement in air.
- b) Flow system with flow cell, the surface of which is modified with carbohydrate and where the signal is transferred with optical, electrochemical, thermal or gravimetric method and where the measuring device is placed in, or in close connection with the cell.
- c) Cuvette or other sample cell, which has been connected with a signal transducer equipped with carbohydrate to which the sample is added.
- d) Planar carbohydrate surface which consists of part of the signal transducer which with ease can be brought into contact with the sample for a suitable time, whereafter the sample is removed and the surface of the signal transducer is characterised with a physical measuring method, for example electronic measurement, gravimetric measurement or thermal measurement.

7.

In some situations, e.g. to increase the biosensor signal in the measurement of low concentrations of cells, it can be advantageous in the measurement of the analyte with the biosensor to add, after the binding of the analyte to the carbohydrate surface, microparticles modified with carbohydrate specific for the bound cell.

The surface of the biosensor can be, for example a gold surface or a modified gold surface, a plastic surface which has been modified with a gold surface, silver surface or another metallic surface, or modifications thereof with polymers to which chemical coupling of carbohydrate can be carried out.

Below are given non-limiting examples of carbohydrate surfaces which can be used in biosensors according to the invention for binding and analysis/determination of pathogenic bacteria of the urinary tract.

EXAMPLE

One example was performed as follows: Silica surface coated with a gold layer was modified with mercaptopropionic acid by dipping the surface in a 5 mM solution of the acid. The carboxyl groups were modified with carbodiimide (EDC) for 2 hours, whereafter digalactoside with aglycon ($\text{Gal}\alpha 1\text{-4Gal}\beta\text{-OEtSEtCONHNH}_2$), was coupled to the EDC-activated surface for 12 hours at pH 8.5 and the surface was then rinsed with buffer.

The thus obtained gold surface modified with digalactoside was dipped for 60 minutes (this time can be varied) in a sample with bacteria of the urinary tract (P-fimbriated *E. coli*) containing $\text{Gal}\alpha 1\text{-4Gal}$ -specific receptor protein, followed by rinsing of the surface with distilled water for 2 minutes. Another gold surface modified in the same way with $\text{Gal}\alpha 1\text{-4Gal}$, was dipped in a sample containing another non-infectious *E. coli* strain which lack the $\text{Gal}\alpha 1\text{-4Gal}$ -specific receptor protein. The extent of binding of the different bacteria to the surfaces was compared with electrom microscopy. The bacteria with The $\text{Gal}\alpha 1\text{-4Gal}$ -receptor bound to the surface to a ca 10-15 times higher extent than the other bacteria. The binding of P-fimbriated *E. coli* to a gold surface modified with mercaptopropionic acid alone, was ca 20 times lower than to the

8.

Gal α 1-4Gal-modified surface.

Alternative non-limiting examples are given below in which a neoglycoprotein was bound covalently or adsorbed directly on a surface for use in biosensor according to the invention.

In procedure B, Gal α 1-4Gal β OCH₂CH₂SCH₂CH₂C(O)-NHNH-BSA was coupled to the same type of EDC-activated gold plate as in the procedure above. The Galabiose-BSA derivative (0.1 mg/ml) was dissolved in 0.1 M boronate, pH 8.5 and EDC-activated plates were immersed in this solution for 1 hour. Subsequently the plates were immersed in a BSA solution (3 mg/ml) in phosphate buffer for 1 minute and rinsed with buffer and distilled water and stored as above.

In procedure C, Gold plates (not pretreated with mercaptopropionic acid) were immersed in a solution of Gal α 1-4Gal β -BSA (0.1 mg/ml) in 0.1 M sodium phosphate, pH 6.0, for 1 hour and subsequently immersed in the above BSA solution (3 mg/ml) for 1 minute, rinsed with buffer and distilled water and stored as above.

These latter biosensor surfaces showed similar characteristics and low back-ground binding of bacteria as surface in the first example above.

CLAIMS

1. Biosensor, characterised in that at least one carbohydrate derivative with ability to bind a protein, virus or a cell in a sample is bound to a surface in the biosensor.
2. Biosensor according to claim 1 above, characterised in that the carbohydrate derivative is chemically bound or is bound via adsorption to a surface which constitutes one part of the biosensors signal transducer part.
3. Biosensor according to claim 1, where the carbohydrate part of the carbohydrate derivative contains at least one component consisting of hexosamine-, fucose-, galactose- glucose-, mannose-, xylose-, N-acetylneuraminic acid residue or an analog thereof.
4. Biosensor according to claim 1, where the carbohydrate part of the carbohydrate derivative contains at least one component consisting of hexosamine-, fucose-, galactose- glucose-, mannose-, xylose-, N-acetylneuraminic acid residue or an analog thereof, which has been derivatised in at least one of their hydroxyl groups or amino groups with an organic or inorganic group.
5. Biosensor according to one or more of the claims above, in which the carbohydrate derivative contains at least one O-, N-, S-, or C-glycosidically bound aglycon.
6. Biosensor according to one or more of the claims above, in which the aglycon part of the carbohydrate derivative contains at least one alifatic or aromatic compound.
7. Biosensor according to one or more of the claims above, in which the aglycon part of the carbohydrate derivative contains an amino acid-, peptide- or protein component.
8. Biosensor according to one or more of the claims above, in which the carbohydrate derivative consist of a glycoprotein or a neoglycoprotein which is bound covalently or via adsorption to a surface which consist of the signal

10.

transducing part of the biosensor.

9. Biosensor according to claim 1, in which the biosensor is an optical biosensor which gives a signal change at the binding of a protein, a virus or a cell to a surface in the biosensor.

10. Biosensor according to claim 9, in which the optical biosensor use surface plasmon changes, ellipsometry, reflection measurement or polarisation measurement.

11. Biosensor according to claim 1, in which the biosensor is based on a piezoelectric crystal, electrochemical electrode or a thermistor.

12. Biosensor according to claim 1, in which the carbohydrate is an oligosaccharide or a derivative thereof which is bound via an aglycon to a surface of the biosensor.

13. Biosensor according to claim 1, in which the carbohydrate is an oligosaccharide or a derivative thereof which is bound via an aglycon to a gold surface of the biosensor.

14. Method to bind a carbohydrate or a derivative thereof to a gold surface, characterised in that the surface first is coated with a thiol compound which contain an organic group which can be used for chemical binding of a carbohydrate or a derivative thereof.

15. Gold surface modified with a carbohydrate or a derivative thereof.

16. Use of biosensor according to claim 1 for determination of or analysis of a protein, a virus or a cell.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 94/00343

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: G01N 33/543, C12Q 1/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOSIS, WPIL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|--|-----------------------|
| X | DE, C2, 3617763 (OLYMPUS OPTICAL CO., LTD.), 17 August 1989 (17.08.89), see fig 13 and page 25 | 1-4,10 |
| Y | -- | 1-16 |
| X | EP, A2, 0215669 (SEIKO INSTRUMENTS & ELECTRONICS LTD.), 25 March 1987 (25.03.87), see p. 6 line 19 and claim 6 | 1-4,10 |
| Y | -- | 1-15 |



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

18 July 1994

Date of mailing of the international search report

29 -07- 1994

Name and mailing address of the ISA/
Swedish Patent Office
Box 5055, S-102 42 STOCKHOLM
Facsimile No. +46 8 666 02 86

Authorized officer

Carl Olof Gustafsson
Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 94/00343

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|---|-----------------------|
| X | WO, A1, 9001166 (ARES-SERONO RESEARCH & DEVELOPMENT LIMITED PARTNERSHIP), 8 February 1990 (08.02.90), see oages 14 and 4, line 19 | 1-4,10 |
| Y | -- | 1-15 |
| Y | WO, A1, 9212995 (MEDOR LABORATORIEN FÜR BIOCHEMIE UND KLINISCHE CHEMIE GMBH), 6 August 1992 (06.08.92) | 1-15 |
| X | US, A, 5082929 (THAT T. NGO ET AL), 21 January 1992 (21.01.92), column 1 - column 2 | 1-4,8 |
| | -- ----- | |

INTERNATIONAL SEARCH REPORT
Information on patent family members

02/07/94

International application No.
PCT/SE 94/00343

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|---|--|
| DE-C2- 3617763 | 17/08/89 | JP-A- 61271458 US-A- 4980278 JP-A- 61271459 | 01/12/86 25/12/90 01/12/86 |
| EP-A2- 0215669 | 25/03/87 | JP-A- 63011835 US-A- 4789804 JP-A- 62207930 JP-A- 62288546 JP-A- 62288547 JP-A- 62064934 | 19/01/88 06/12/88 12/09/87 15/12/87 15/12/87 24/03/87 |
| WO-A1- 9001166 | 08/02/90 | AU-B- 638938 AU-A- 4043389 DE-D- 68914134 EP-A- 0353937 | 15/07/93 19/02/90 00/00/00 07/02/90 |
| WO-A1- 9212995 | 06/08/92 | DE-A- 4101394 | 23/07/92 |
| US-A- 5082929 | 21/01/92 | NONE | |

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☒ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.